ESR OF Cu^{2+} BOUND TO POLYNUCLEOTIDES AND DERIVATIVES

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Binding of Cu^{2+} to polynucleotides has been found to occur at 1 Cu/5 polymer P and at similar molar ratios of copper to selected nucleic acid derivatives. Electron spin resonance (ESR) measurements show that the *g*-values and the hyperfine splitting constants vary substantially among the different complexes. Binding of Cu^{2+} to the phosphates as well as to the bases is observed, and in some cases occurs simultaneously.

1. Introduction

Cu^{2+} binding to nucleosides, nucleotides and deoxyribonucleic acid has been studied in the recent years by various authors using diverse biochemical and physical approaches [1]. It has been concluded that Cu^{2+} binds in the above systems to the phosphate groups and/or to the nitrogens of the bases. The latter type of bond manifests itself, among other things, in the drastic lowering of the melting temperature of DNA [2].

We have measured the electron spin resonance (ESR) parameters of Cu^{2+} bound to nucleosides, nucleotides and polynucleotides in order to further elucidate the characteristics of these complexes.

2. Materials and methods

Calf thymus DNA was obtained from Worthington Biochemical Corp., the *M. Lysodeikticus* DNA, the homopolyribonucleotides, dG:dC and poly d(A–T) were purchased from Miles Laboratories, Inc. The bases, deoxynucleosides and deoxynucleotides were obtained from Sigma Chemical Corp. The solutions were prepared unbuffered at concentrations of 2 mg/ml in 5 mM NaCl and mixed at room temperature with $CuSO_4$ solution of proper molarity to obtain the ratio of 1 Cu^{2+} to 5 polymer P, or 1 Cu^{2+} to 5 bases, nucleosides, or nucleotides. This 1:5 ratio was varied in only one set of experiments as described in the text.

The ESR was measured on a Varian V-4500 spectrometer at a frequency of around 9200 MHz, at liquid nitrogen temperatures. The magnetic field was measured with the Varian F-8A flux-meter and klystron frequency was determined with Hewlett-Packard Transfer Oscillator, model 540 B. The spectra are described specifying the *g*-values and the hyperfine constants. Only the hyperfine splitting in the parallel direction has been observed, yielding $g_{||}$, and A. $g_{\perp}$ is not well defined in the frozen solutions and we determined instead the *g*-values at maximum absorption, g_m, which is very close to $g_{\perp}$ [3].

3. Results and discussion

All the results are summarized in table 1 and fig. 2. Table 1 summarizes the values of $g_{||}$, g_m and A obtained for all compounds. Fig. 1a shows a typical spectrum of a Cu^{2+} complex exhibiting a single set of the hyperfine lines. Fig. 1b shows the expanded spectrum of the low field region of the same complex and fig. 1c shows the expanded spectrum for a complex with two hyperfine sets of lines. The presence of two sets of lines is also indicated in table 1 and fig. 2.

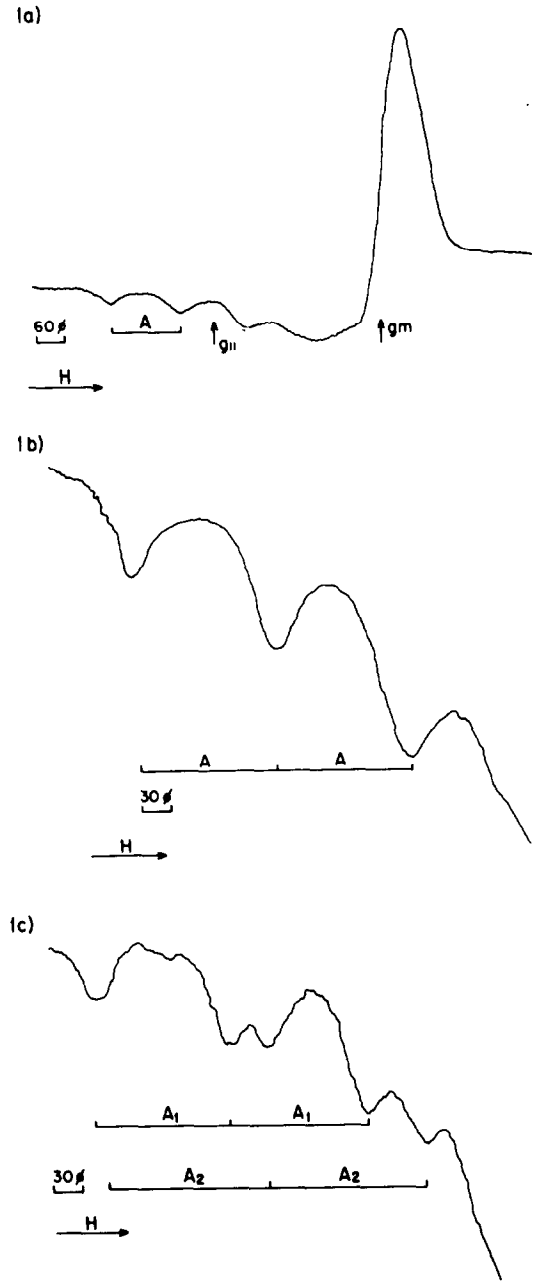

Fig. 1. (a) ESR Cu^{2+} spectrum of adenosine-Cu^{2+} complex. A/Cu = 5, A at 2 mg/ml, pH 5.5, T = 77° K.
(b) Low field, expanded portion of the above spectrum. One set of hyperfine lines indicates the presence of a single binding site for Cu^{2+}.
(c) Low field, expanded spectrum of d(A–T)-Cu^{2+} complex. 1 Cu/5 polymer P, pH 5.5, T = 77° K, d(A–T) at 2 mg/ml.

Table 1
Electron spin resonance parameters of various Cu^{2+} complexes at T = 77° K and pH 5.5.

	g_m	$(g_{\|\|})_1$	$(g_{\|\|})_2$	$A_1(\phi)$	$A_2(\phi)$	g
Cytidine	2.050	2.26		175		2.120
CMP	2.080	2.38		135		2.180
Thymidine	2.081	2.39		130		2.184
TPM	2.090	2.37		142		2.183
Guanosine	2.09	2.32		162		2.166
GMP	2.122	–				
Adenosine	2.080	2.35		143		2.170
AMP	2.080	2.36		135		2.173
Uridine	2.097	2.40		130		2.198
UMP	2.083	2.37		150		2.178
DNA(C.T.)	2.072	2.36	2.32	140	170	2.168, 2.154
DNA (M.L.)	2.07	2.37		141		2.170
d(A–T)	2.083	2.34	2.28	145	165	2.155, 2.142
dG:dC	2.075	2.36		140		2.170
Poly C	2.067	2.34	2.27	179	154	2.158, 2.134
Poly G	2.082	2.33		154		2.164
Poly A	2.086	2.34		156		2.170
Poly U	2.085	2.41		125		2.193
2-Deoxy-D-ribose	2.092	2.40		135		2.194
$CuSO_4$	2.175					

$(g_{||})_1$, $(g_{||})_2$ and $A_1(\phi)$, $A_2(\phi)$ refer to two Cu^{2+} sites in some complexes. $A_1(\phi)$ and $A_2(\phi)$ in gauss; $g = \frac{1}{3}(2g_m + g_{||})$, $g_{||}$, and A indicated in fig. 1a.

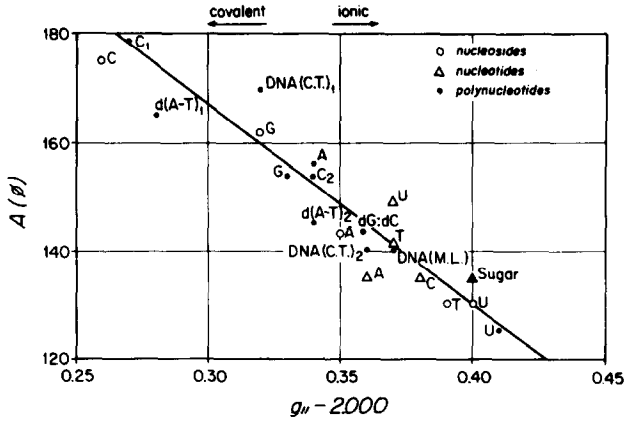

Fig. 2. A plot of the hyperfine constant A vs $g_{||} - 2.000$ for Cu^{2+} complexes with polynucleotides and derivatives. 1 Cu^{2+}/5 polymer P, 2 mg/ml, pH 5.5, T = 77° K.

We have run two sets of controls for the present experiments. In order to determine whether we are actually dealing with Cu^{2+} ions bound to the different complexes we have measured the parameters of $CuSO_4$ frozen solutions. Its $g_m = 2.175$ and there was no indication of resolved hyperfine structure. Since none of the copper complexes studied here has these characteristics it can be concluded that in the conditions of our experiments practically all Cu^{2+} was bound. This is not necessarily so at higher ratios of Cu^{2+} to the molecules. In the case of CMP, for instance, on varying this ratio (and keeping the CMP concentration constant) between 1:10 and 2:1, a gradual broadening of the line, from 110 to 210 gauss, and a shift of g_m from 2.0803 to 2.16 is obtained. This is undoubtedly due to the mixture of bound and unbound Cu^{2+} in the sample at higher copper molarity. In order to obtain pure spectra of the complexes we have chosen 1:5 (Cu/molecule) ratios for all our experiments.

In order to check whether the compounds used in the present series were denatured, we have performed a series of optical measurements. The absorption at 260 nm was measured at room temperature in DNA, d(A–T), dG:dC and polyribonucleotides with Cu^{2+} concentrations corresponding to those used in the ESR series. A control series was run without added Cu^{2+}. In all cases the absorption with an without Cu^{2+} was identical to within ± 1%. The samples were then heated to 100° and quenched at 0°. All samples showed hyperchromicity of minimum 6% in the case of dG:dC and poly U, to a maximum of almost 100% in the case of d(A–T). The hyperchromicity for samples with an without Cu^{2+} was identical to ±2%. We conclude therefore that the polynucleotides were not denatured at room temperature in the presence of Cu^{2+}.

The Cu^{2+} spectra in table 1 exhibiting 2 sets of hyperfine lines are interpreted as due to two non-equivalent sites in each molecule, each one characterized by its own set of g-values and a different value of A.

The interpretation of Cu^{2+} ESR spectra, in general, follows two lines. Kivelson and Neiman [4] have correlated the changes in the magnitudes of A and g with changes in covalency of the Cu^{2+} bonds. Using this approach we have constructed fig. 2 from the data in table 1. Blumberg [5] has used a different approach and examined ESR and optical data of Cu proteins in terms of distortion of the copper surrounding octahedra.

The plot of fig. 2 indicates a considerable spread in the magnitudes of the observed ESR parameters of Cu^{2+} in the various complexes. We feel that it is premature to interpret these data in detail. Some trends are however suggestive. It is apparent for instance, in comparing the results obtained on the various cytosine complexes, that poly C exhibits two binding sites (C_1 and C_2), while cytosine only a single one of characteristics similar to C_1. This suggests that C_1 corresponds to a site on the base while C_2 to a phosphate. It is not surprising that the former is of a more covalent nature. The NMR evidence suggests that this site corresponds to the position N_1 of cytosine [1].

The double helical structure of d(A–T) also binds Cu at two sites. By analogy with the cytosine compounds the phosphates and the bases are implicated. On the other hand, dG:dC exhibits only a single site, indicative of a Cu to phosphate bond. In this polynucleotide the position N_1 of cytosine is occupied in hydrogen bonding to guanine.

The results of DNA show that while calf thymus DNA exhibits two sets of hyperfine lines (two sites), the M.L. DNA shows only one, with characteristics corresponding to the phosphate bond. The difference may be due to the fact that C.T. DNA has only 44% of G–C pairs while M.L. DNA 67%. The position N_1 of cytosine (as well as positions 2 and 6) are involved in hydrogen bonds hence binding of Cu to the bases of the A–T pairs (more abundant in C.T. DNA) may be responsible for the difference.

We observe, in conclusion, consistent effect of binding of Cu^{2+} to A–T pairs of a double helix. This is in apparent contradiction to the results of various authors [2, 6]. We have to stress, however, that the present experiments are performed with a lower Cu/P ratio than most other investigations, and at higher concentrations of the polynucleotides. The relatively low Cu^{2+} concentrations used in the present experiments correspond more closely to the physiological conditions. It is not excluded that at higher Cu/P ratios or at higher ionic strength, binding to the G–C pairs also occurs.

References

[1] G.I.Eichhorn, P.Clark and E.D.Becker, Biochemistry 5 (1966) 245.
[2] S.Hiai, J. Mol. Biol. 11 (1965) 672.
[3] B.G.Malmstrom and T.Vanngard, J. Mol. Biol. 2 (1960) 118.
[4] D.Kivelson and E.Neiman, J. Chem. Phys. 35 (1961) 149.
[5] W.Blumberg, in: The Biochemistry of Copper, eds. Peisach et al. (Academic Press, New York, 1966) p. 49.
[6] Ch.Zimmer and H.Venner, Studia Bioph. 2 (1967) 207.